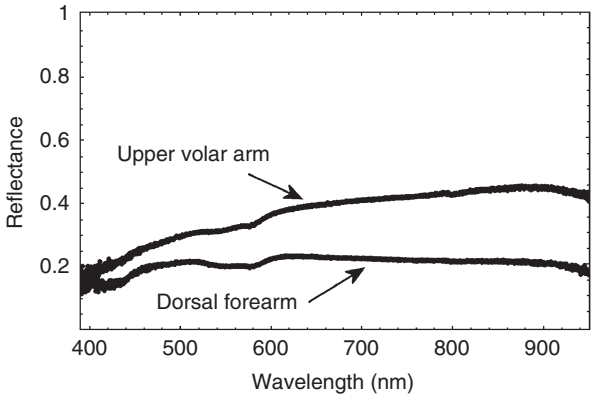

Figure 2. Reflectance measurements after correcting for detector dark current and for the light source spectrum.

technique, such as the one presented in Eq. (1), should be used.

Figure 2 shows the same reflectance measurements of Figure 1 after correcting for the detector dark current and for the light source spectrum. The spectra shown in Figure 2 closely resemble previously published data (Takiwaki *et al.*, 2004; Matts *et al.*, 2007) taken with different instrumentation setups.

In summary, RS can be used as a diagnostic aid in dermatology if proper correction and normalization techniques are employed.

CONFLICT OF INTEREST

The author states no conflict of interest.

ACKNOWLEDGMENTS

I acknowledge fruitful discussions with Benjamin Moncada from the Dermatology Department, Hospital Central, San Luis Potosi, SLP, Mexico, and technical assistance from Isaac Muñoz and Edgar Guevara in the acquisition and processing of reflectance spectra.

***Francisco J. González*[1]**

[1]*Instituto de Investigación en Comunicación Óptica, Universidad Autónoma de San Luis Potosí, San Luis Potosi, SLP, Mexico*
E-mail: javier@cactus.iico.uaslp.mx

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Response to González

Journal of Investigative Dermatology (2009) **129,** 1583–1584; doi:10.1038/jid.2008.437

TO THE EDITOR

The author is correct in his statement that appropriate use of a reflectance spectrophotometer requires an instrument setup with 100% transmission standards (white) and 0% transmission standards (black or dark current). Indeed, as stated in the Materials and Methods section, these standards, as well as Labsphere ISO 9901-certified reflectance standards red, green, yellow, and blue, and neutral color standards 20, 50, and 99% transmission standards were evaluated in the study exam each day before the evaluation of a human being, and repeated every 2 hours throughout each study session to insure optimal instrument quality assurance. Values for all standards demonstrated temporal variability of less than 10% in a dedicated study exam room with no windows, and controlled ambient temperature ($23 \pm 2^{\circ}C$) and relative humidity (25–30%). To our knowledge, such quality assurance methods have neither been incorporated nor been validated in earlier studies.

Abbreviations: ISO, international organization for standardization; UVR, ultraviolet radiation

The novelty of the current reflectance spectrophotometer method to establish a skin phototype compared with previously published methods is the use of a single and readily accessible photo-protected skin site at the upper volar arm to establish a baseline skin color. Although a photo-exposed site is often used to assess the ability of skin to ''tan'' or quantify UVR exposure history, it was not used in the current studies to establish skin phototype owing to its demonstrated seasonal dependence.

The instrument light source noted by González, as well as spectral digitizing

software, room temperature, ambient light conditions, and probe angle may influence the reflectance spectral values. Thus, all reflectance spectrophotometers should be independently validated in appropriate and known Fitzpatrick skin types under the environmental and investigator conditions of assessment to establish the mathematical-fitted equation between subjective and objective skin phototype. Given that the same light source was utilized throughout the studies, and reflected light spectra of commercial color and light/dark standards were validated daily, normalization of the reflectance spectral data with the dark current (0% transmission) and light source spectrum before each subject was not necessary for an accurate skin phototype assessment. Normalization methods may be useful, however, when comparison between instruments, light sources, investigators, or study locations is desired.

Lynn K. Pershing[1], Laurence J. Meyer[1] and Sancy L. Leachman[1]

[1]Department of Dermatology, University of Utah School of Medicine, Salt Lake City, Utah, USA E-mail: lynn.pershing@hsc.utah.edu

Independent Evaluation of a Commercial Test for ''Autoimmune'' Urticaria in Normal and Chronic Urticaria Subjects

Journal of Investigative Dermatology (2009) **129,** 1584–1586; doi:10.1038/jid.2008.416; published online 8 January 2009

TO THE EDITOR

Although the pathogenesis of the majority of chronic idiopathic urticaria (CIU) patients is unknown, approximately 40% of patients are proposed to have ''functional'' IgG autoantibodies against either the high-affinity IgE receptor (FcεRIα) or IgE as measured by the basophil histamine-releasing activity (HRA) assay (Ferrer and Kaplan, 2007). This assay involves incubating basophils from a healthy donor with a CIU patient's serum and measuring histamine release. A ''positive'' result is often judged in relation to basophil HRA levels obtained with serum from a non-CIU population. Some investigators have proposed that the presence of ''functional'' autoantibodies offers a pathogenic explanation for patients' symptoms or provides a rationale for treatment with immunomodulatory therapy such as cyclosporine (Sabroe and Greaves, 2006). However, the HRA assay, which is considered the ''gold standard'' (Ferrer and Kaplan, 2007) for ''functional'' autoantibodies, has limitations. Its performance depends on the unique characteristics of the healthy basophil donors (Eckman *et al.*, 2008). In addition, interlaboratory reproducibility of this test has not been possible to assess because of a lack of universally available standardized reagents.

An alternative line of investigation has shown that basophils from active CIU subjects manifest a suppressed IgE receptor-mediated histamine degranulation (Ferrer and Kaplan, 2007). We have stratified CIU subjects into responder (CIU R) and nonresponder (CIU NR) functional phenotypes based on the profile of *ex vivo* activation of their basophils by an optimal concentration (0.1 $\mu g\,ml^{-1}$) of polyclonal anti-IgE (Vonakis *et al.*, 2007). Subjects with $<10\%$ histamine release with anti-IgE stimulation were classified as CIU NR, whereas subjects with histamine release $\geqslant 10\%$ were classified as CIU R. This basophil classification remains consistent over the course of active disease (Eckman *et al.*, 2008). Interestingly, increased basophil IgE-receptor-mediated histamine release occurs in both groups as subjects enter disease remission (Eckman *et al.*, 2008). Using a previously identified immunoenzymometric assay (IEMA), we observed a similar prevalence and concentration of IEMA-detected autoantibodies in the CIU R, CIU NR, and non-CIU subject groups (Eckman *et al.*, 2008). We have previously reported that positive HRA presence occurs at a similar frequency in CIU R, CIU NR, and nonatopic, healthy (normal) subjects (Vonakis *et al.*, 2007). The positive HRA results we obtained in the non-CIU subjects have been challenged (Kaplan and Joseph, 2007).

The purpose of this study was to assess HRA activity in sera from CIU and non-CIU subjects by the CU Index test by IBT Laboratories (Lenexa, KS). Further, we examined the relationship of HRA activity to previously determined CIU basophil classification and the presence of IEMA-detected autoantibodies.

Following consent, whole-blood was collected for basophil and serology studies from subjects with a physician-determined diagnosis of CIU ($n=21$) or non-CIU controls ($n=22$; ages 18–65, 12 female, 10 male, 9 atopic, 13 nonatopic by history). None of the non-CIU subjects had any known autoimmune disease. Protocols were approved by the Johns Hopkins Institutional Review Board and the Western Institutional Review Board and were in adherence to the Declaration of Helsinki Principles (Eckman *et al.*, 2008). Basophil functional phenotyping studies were performed as described

Abbreviations: CIU NR, CIU non-responder; CIU R, CIU responder; CIU, Chronic idiopathic urticaria; HRA, Histamine release activity; IEMA, Immunoenzymetric assay